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Immunological defects in SJL mice

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SUMMARY

SJL mice are shown to be defective in their ability to develop suppressor cells following stimulation with Con A, a polyclonal T-cell activator. They make a normal proliferative response to this mitogen. In addition to this suppressor T-cell defect, the SJL mouse (unlike most mouse strains) does not develop a spontaneous antibody response to bromelain-treated autologous red blood cells (BrMRBC) *in vitro*. Although the SJL makes a normal proliferative response to LPS, antibody-forming cells against bromelain-treated autologous red blood cells are not increased following LPS *in vivo* nor does it manifest an increased response to SRBC or TNP. This may signify the presence of a functional B-cell defect in these animals. DBA mice are also shown, in this report, to have small numbers of antibody-forming cells to bromelain-treated autologous red blood cells but to be capable of responding to LPS *in vivo* with an increase in SRBC and TNP antibody responses.

INTRODUCTION

The SJL mouse has been shown to be extremely sensitive to the induction of autoantibodies and autoimmune disease (Vladutiu & Rose, 1971; Brown & McFarlin, 1981). This sensitivity may be related to the known SJL defect in those suppressor cells involved in the maintenance of tolerance (Amagai & Cinader, 1981) and regulation of erythrocyte autoantibodies (Cooke & Hutchings, 1984).

It has been shown that when normal murine spleen cells are stimulated with a supraoptimal dose of concanavalin A (Con A) non-specific suppressor T cells are generated. (Dutton, 1973; Rich & Pierce, 1973). We have extended our previous observations in the SJL mouse to a study of these non-specific suppressor cells generated following polyclonal T-cell activation.

The second part of our study concerns a further defect in the SJL mouse which appears to be independent of the T suppressor cell abnormality.

When peritoneal cells or spleen cells are cultured for 3 days, a spontaneous increase in the number of antibody-forming cells with specificity for bromelain-treated mouse red blood cells (Br.MRBC), the so-called autologous plaque-forming cells, is observed (Pages & Bussard, 1975). An anti-idiotype has been raised by Pages *et al.* (1982) to monoclonals with specificity for Br.MRBC. Using the anti-idiotype, these workers have demonstrated that this idiotype was not detectable in the sera of SJL and DBA mice (Poncet *et al.* (1985). Since it has already been demonstrated that some T suppressor cells are defective in SJL mice and since this T-independent antibody response to Br.MRBC can be induced by LPS in normal mice and has been shown to be regulated by T suppressor cells (Cunningham, 1975; Cox *et al.*, 1979) we therefore investigated whether the response to Br.MRBC was really reduced or whether it was actively suppressed in SJL and DBA mice.

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MATERIALS AND METHODS

Mice

All mice used were between 8 and 12 weeks of age. SJL mice were bred in our own animal house, the original breeding stock having been purchased from OLAC 1976 Ltd (Shaws Farm, Blackthorn, Bicester, Oxon). CBA and (CBA × BALB/c)F_1 mice were purchased from the NIMR (The Ridgeway, Mill Hill, London).

Preparation of bromelain-treated mouse RBC (Br MRBC)

Mouse RBC were washed three times with phosphate buffered saline (PBS) before treatment with the enzyme bromelain (Sigma Chemical Co., St Louis, MO). Bromelain-treated mouse RBC (Br.MRBC) were prepared according to the method of Cunningham (Cunningham, 1974). Briefly, 1 ml of packed mouse RBC was incubated with 1 ml of a bromelain solution in PBS (10 mg/ml final concentration) for 40 min at 37°. The cells were washed three times in PBS before use in the plaque assay.

Plaque-forming cell (PFC) assay

TNP was coupled to SRBC as described by Rittenberg & Pratt (1969). TNP, SRBC and Br.MRBC plaque assays were carried out using Cunningham chambers (Cunningham & Szenberg, 1968) and guinea-pig complement.

Generation of suppressor cells with Con A

Spleen cells were cultured at 10^7/ml for 48 hr with Con A (Miles Lab., Slough, Berks.) at 2, 4 or 8 μg/ml in RPMI-1640 containing 5% fetal calf serum, 5 mM glutamine, 100 units/ml Penicillin G and 100 μg/ml streptomycin, 5×10^{-5}/mM 2-mercaptoethanol. All cultures were carried out at 37° in 5% CO_2 and air.

Suppressor cell assay

Sheep RBC (SRBC) primed spleen cells (3×10^6/well) were cultured for 5 days in the presence or absence of suppressor cells (1×10^6/well). Cell cultures were carried out in Linbro plates in a volume of 2 ml in RPMI-1640 and supplements as above. SRBC were included in the cultures as antigen at a final concentration of 10^6/well. Cultures were harvested after 5 days and assayed for SRBC plaque-forming cells.

Response to Br.MRBC

Peritoneal cells (2×10^5) were cultured in 200 μl RPMI, 5% FCS and supplements as above in microtitre plates for 3 days. At Day 0 in normal mice the plaque response is zero but by Day 3 and even as early as Day 1 large numbers of cells making antibody against Br.MRBC can be detected. *In vivo* responses were measured 3 days after an injection i.p. of 20 μg lipopolysaccharide (LPS) or saline.

Mitogen assay

Spleen cells were cultured at 2×10^6/ml in 200 μl aliquots in microtitre plates with mitogen for 48 hr, pulsed overnight with ^{125}I-Udr (0·5 μCi/well, Amersham International, Berks.) and harvested on an automated cell harvester.

Statistics

Statistical analysis of data was carried out using a Student's *t*-test.

RESULTS

Suppressor cells are not developed in SJL mice following Con A stimulation

SJL spleen cells, and for comparison, CBA spleen cells, were activated for 48 hr with Con A (2, 4 or 8 μg/ml) and assayed for their ability to suppress an *in vitro* secondary response to SRBC. From Fig. 1 it can be seen that CBA spleen cells develop suppressor activity following such culture whereas SJL spleen cells do not respond to Con A stimulation with the generation of suppressor cells. This was true at all the concentrations of Con A tested. Nevertheless the SJL mouse does give a mitogenic response to Con A (Table 1). SJL and ASW spleen cells (both H-2^s) were activated for 48 hr with Con A (4 μg/ml) and the putative suppressor cells were then assayed for their ability to suppress the *in vitro* secondary response to SRBC of either SJL or ASW spleen cells. It was found that the SJL cells could suppress neither response, whereas the ASW cells could suppress the syngeneic response but not the SJL. The ASW responses were very poor compared with the CBA and SJL but the suppression by syngeneic cells was significant (Table 2).

Response to bromelain-treated mouse RBC

When (CBA × BALB/c)F_1 peritoneal cells are cultured for 72 hr

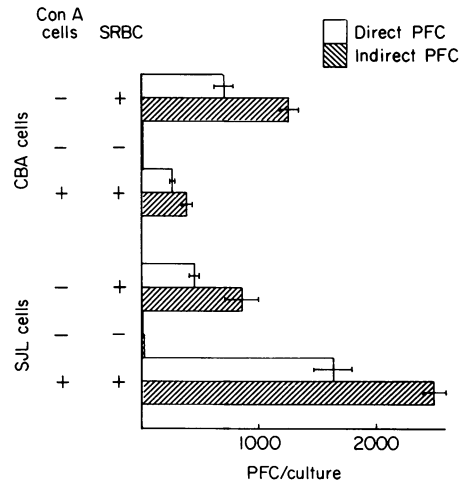

Figure 1. Con A suppression assay. Primed spleen cells (3×10^6) were cultured for 5 days ± 1×10^6 SRBC. Syngeneic suppressor cells (3×10^6), generated by 48 hr culture with Con A 4 μg/ml, were added where indicated. Results are the mean PFC against SRBC of triplicate cultures ± SE.

Table 1. Response to mitogens by CBA and SJL spleen cells

Mitogen	CBA	SJL
Media	3909 ± 331	1545 ± 43
Con A (2 μg/ml)	97,070 ± 11085	41,131 ± 1362
LPS (40 μg/ml)	21,143 ± 901	19,424 ± 1591

Mean of triplicate wells c.p.m. ± SE. 4×10^5 spleen cells ± mitogen were cultured for 48 hr and then pulsed overnight with ^{125}I-Udr.

Table 2. Effect of SJL or ASW suppressor cells on secondary *in vitro* SRBC responses

SRBC	Suppressor cells	Direct anti-SRBC PFC/culture Day 5 primed spleen cells: CBA	SJL	ASW
−	−	50 ± 50	283 ± 161	17 ± 12
+	−	10,117 ± 1640	7417 ± 2109	310 ± 26
+	CBA	1967 ± 252	ND	ND
+	SJL	ND	5700 ± 737	287 ± 21
+	ASW	ND	5917 ± 666	*157 ± 25

Primed spleen cells (3×10^6) were cultured for 5 days ± 10^6 SRBC. 10^6 suppressor cells generated with Con A (4 μg/ml) were added where indicated. Mean PFC in triplicate wells ± SE.

* Significantly suppressed, $P < 0.02$.

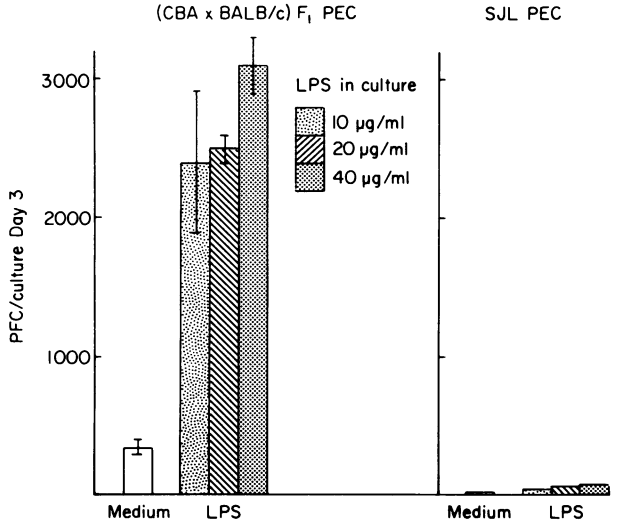

Figure 2. SJL PEC do not make a response against Br.MRBC and do not respond to LPS *in vitro*. PEC (2×10^5) were cultured for 3 days ± LPS as indicated. PFC against Br.MRBC were assayed on Day 3 and results are the mean of triplicate cultures ± SE.

antibody-forming cells can be demonstrated with specificity for Br.MRBC. This response is enhanced by the addition of LPS (40 μg/ml) at Day 0 (Fig. 2). SJL peritoneal cells do not respond in this manner even when LPS is added to the culture (Fig. 2). In normal mice this response is known to be subject to T-cell suppression (Cunningham 1975; Cox *et al.*, 1979). T-cell depletion of spleen cells with anti-Thy 1.2 and complement did not restore the bromelain response in SJL mice (Table 3) therefore the lack of response in this mouse is not due to overriding T-cell suppression.

Autologous plaque-forming cells are also increased when normal spleen or peritoneal cells are cultured with PPD. PPD does not however stimulate autologous plaque formation by SJL spleen or peritoneal cells (Table 4).

Autologous plaque-forming cells can also be demonstrated *in vivo* in the spleens of normal mice 3 days after i.p. injection of LPS (20 μg). The background responses to SRBC and TNP are similarly increased by such polyclonal B-cell activation. The typical response of normal mice to LPS is shown in Fig. 3. It can be seen from Fig. 3 that SJL mice fail to show increased

Table 4. Defective responses to Br.MRBC are not enhanced in SJL by PPD or LPS

		PFC/culture Day 3			
			LPS	PPD	
		Medium	40 μg/ml	100 μg/ml	300 μg/ml
PEC	CBA	720±121	>5000	990±14	1890±141
	SJL	13±6	40±10	3±6	13±12
Spleen	CBA	197±6	463±65	590±30	480±100
	SJL	0	7±6	36±35	0

2×10^5 peritoneal exudate cells were cultured with LPS or PPD at the final concentrations shown and the wells were harvested as above.

responses to TNP, SRBC or Br.MRBC following LPS *in vivo*. Furthermore, when LPS stimulated or normal SJL and CBA mice were examined for total IgM PFC (reverse plaques) the LPS stimulated CBA showed a 10-fold increase over the normals, whereas the very low numbers in unstimulated SJL mice were not increased by LPS (D. W. Dresser, personal communication).

This lack of response of SJL mice to LPS is not attributable to an inability to respond to LPS as a mitogen since these mice gave a normal proliferative response to LPS (Table 1).

Bussard, Pages & Poncet (1984) and Poncet *et al.* (1985) have reported that they were unable to detect an idiotype that is present on antibodies to Br.MRBC in the sera of SJL and DBA mice. This idiotype has been found in the serum of all other adult strains of mice studied. Therefore, we examined the responses of DBA mice to Br.MRBC and their ability to respond to LPS and compared these observations to those obtained from the SJL mouse. From Table 5 it can be seen that the DBA mouse also appears to have low numbers of autologous PFC but, unlike the SJL, the numbers of PFC to Br.MRBC, SRBC and TNP-SRBC are increased by LPS. However, the increased number of PFC to Br.MRBC in DBA peritoneal cells after culture with LPS is not so marked as that seen using CBA or (CBA × BALB/c)F_1 peritoneal cells (Table 3) which may reflect a much smaller precursor pool. As in the SJL, treatment of DBA PEC with anti-

Table 3. Defective responses to Br.MRBC are not restored by treatment with anti-Thy 1.2

	PFC/culture Day 3					
	(CBA × BALB/c)F_1		DBA		SJL	
LPS	Untreated	Anti-Thy 1.2	Untreated	Anti-Thy 1.2	Untreated	Anti-Thy 1.2
−	152±22	212±34	0	2±3·5	24±0	20±14
+	1078±292	1198±73	16±3·5	38±9	26±12	26±9

2×10^5 T-cell depleted or normal PEC were cultured ± LPS (40 μg/ml) and harvested on Day 3. Mean of triplicate wells ± SE.

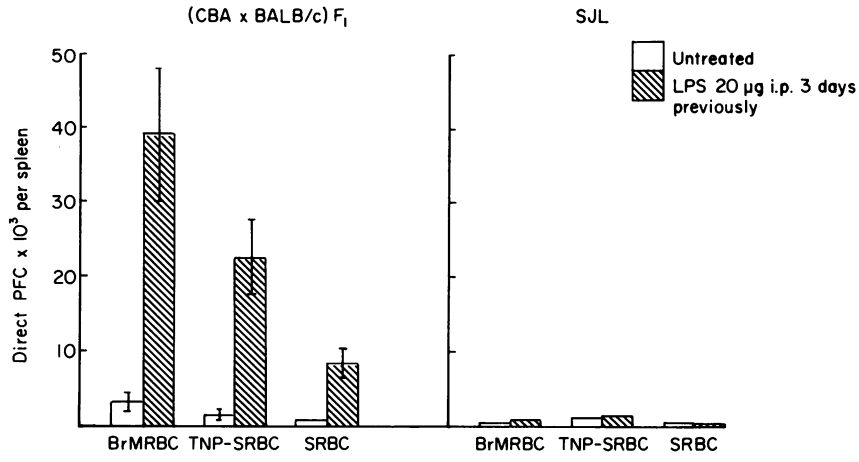

Figure 3. SJL spleen cells do not respond to LPS *in vivo*. Spleens of mice given 20 μg LPS i.p. were assayed after 3 days for the number of PFC against Br.MRBC, TNP-SRBC and SRBC. Results are the mean ± SE, four mice in a group.

Table 5. Effect of LPS on background IgM responses *in vivo* in CBA and DBA mice

	PFC/spleen Day 3					
	CBA			DBA		
LPS	Br.MRBC	SRBC	TNP-SRBC	Br.MRBC	SRBC	TNP-SRBC
20 μg i.p.	48,400 ± 8627	2233 ± 1137	6,900 ± 1249	1666 ± 611	733 ± 321	6900 ± 1493
—	2066 ± 1069	300 ± 100	1033 ± 305	200 ± 100	67 ± 57	733 ± 115

Mean ± SE. Three mice in a group.

Thy 1.2 + GPC failed to show that the poor response was due to T-cell suppression (Table 3).

CBA/N mice do not respond to Br.MRBC or to some T-independent antigens (TI-2). To test the possibility that the SJL, like the CBA/N is unable to respond to TI-2 antigens, these mice were immunized i.v. with TNP-Ficoll. Five days later, their spleens were assayed for numbers of antibody-forming cells against TNP-SRBC. Table 6 shows that the SJL mouse can respond normally to TNP-Ficoll.

Table 6. Response *in vivo* of SJL mice to TNP-Ficoll

Anti-TNP	PFC/spleen	Day 5
	CBA	SJL
75 μg TNP-Ficoll i.v.	39,750 ± 5615	24,300 ± 11,980
—	11550 ± 212	400 ± 282

Mean ± SE. Four mice in a group.

DISCUSSION

It has been shown that SJL mice do not develop those suppressor cells which play a role in the maintenance of tolerance to both heterologous and self antigens (Amagai & Cinader, 1981; Cooke & Hutchings, 1984). This may contribute to the ease of induction of autoantibodies and autoimmune disease in these animals (Vladutiu & Rose, 1971; Brown & McFarlin, 1981; Cooke & Hutchings, 1984). In this paper we have extended these observations on suppressor cell defects in SJL mice to another experimental system that generates so called 'non-specific' suppressor cells. This function also appears to be defective in the SJL and, moreover, preliminary experiments suggest that the targets of suppression in this mouse may also be refractory in this system.

The only pathology that manifests itself in some SJL mice is the spontaneous occurrence of neoplasia that have either been described as reticulum sarcomas or B-cell lymphomas (McIntire & Law, 1967; Ford, Ruppert & Maizel, 1981). These animals do not spontaneously develop any autoimmune disorder suggesting that suppressor cell defects alone do not necessarily predispose one to the spontaneous occurrence of autoimmune disease.

An unexpected finding in this mouse strain was the inability to develop a response to Br.MRBC either *in vitro* or *in vivo*

following polyclonal B-cell activation. This could be explained in several ways: (a) restriction of B-cell responsiveness due to a change in the frequency of light chain isotypes; (b) lack of a specific B-cell subset or (c) inability to respond to certain B-cell differentiative signals.

These possibilities are not mutually exclusive and each will be discussed in turn.

Restriction of B-cell responsiveness due to lack of light chains

The SJL mouse is known to differ from other strains in the level of λ light chains in normal immunoglobulin, the λ gene in the SJL being subject to regulation such that the serum level of λ is diminished 50-fold (Geckeler *et al.*, 1977). Therefore, if the Br.MRBC response is, like the response to α (1–3) dextran, predominantly λ, a defect would appear to be present in the response to LPS. However, the response to Br.MRBC cannot be wholly λ since the monoclonal antibodies to Br.MRBC isolated by Poncet *et al.* (1985) have κ light chains.

Lack of a specific B-cell subset

The CBA/N mouse does not make responses to some T-independent antigens, for example TNP-Ficoll which is classified as a T1-2 antigen. In addition, although most mouse strains do have PFC to Br.MRBC, these have been shown to be absent in the CBA/N (Jayawardena, Janeway & Kemp, 1979; Marshall-Clarke, Cooke & Hutchings, 1979). Although the SJL mouse does not make a response to Br.MRBC, it does respond to TNP-Ficoll (Table 5), indicating that it does not carry a defect entirely analogous to the CBA/N. The response to Br.MRBC has been shown to reside in the Ly-1 B-cell pool (Hayakawa *et al.*, 1984) whereas the response to TNP-Ficoll or TNP-KLH is found in the B-cell pool which does not bear the Ly-1 marker. Since SJL mice respond to TNP-Ficoll and TNP-KLH but not to Br.MRBC they may lack functional Ly-1 B cells. In support of this is the observation of Hayakawa *et al.* (1984) and Poncent *et al.* (1985) that there are 20–40% Ly-1 B cells in the peritoneal cavity of normal mice but only 1–5% in the peritoneal cavity of SJL and SJA mice.

An anti-idiotype has been raised to monoclonal antibodies to Br.MRBC which can be detected in the serum of all strains of adult mice except SJL and DBA (Poncet *et al.*, 1985). In this context, our data (Table 3 and Table 5) suggest that the DBA mouse may also have a defect in the autologous plaque response although in the DBA, unlike the SJL, this reduced response to Br.MRBC, together with the response to SRBC and TNP can be stimulated by LPS. Dresser (1982) showed that the total IgM PFC in the DBA, although fewer, were increased with LPS. In this case, the response to LPS is normal but the responding Ly-1 B-cell pool is much smaller.

Additionally, there is substantial evidence demonstrating that Br.MRBC are antigenically cross-reactive with some epitopes on SRBC (Pages & Bussard, 1975). This means that some of the B cells responsive to SRBC also react with Br.MRBC. Therefore, failure to demonstrate an increased Br.MRBC or SRBC response in SJL mice following LPS stimulation may reflect that these responses derive from the same B-cell pool and that this population is reduced in the SJL. However, this cannot be the whole explanation for the unresponsiveness in SJL mice since the starting responses to Br.MRBC and SRBC are similarly reduced in DBA mice but they can be stimulated normally by LPS whereas these responses cannot be expanded in the SJL.

Inability to respond to certain B-cell differentiation signals

Goodman & Weigle (1985) have shown that B lymphocytes from SJL mice, unlike other mice, are unable to respond to early stimulatory signals provided by C8 substituted guanine ribonucleosides. These mice can, however, respond to the late differentiative signals also provided by these ribonucleosides. In the Goodman & Weigle (1985) experiments, no proliferative response was seen to these ribonucleosides which they interpret as being related to the unresponsiveness to early signals. In our experiments using LPS, SJL mouse spleen cells proliferative to LPS but do not develop a differentiated B-cell response. This suggests that the defect we observed in the SJL is not equivalent to that described by Goodman & Weigle (1985).

C3H/HeJ mice do not respond to LPS since they lack a receptor for LPS (Skidmore *et al.*, 1975). The ability of the SJL to respond mitogenically to LPS while not showing an increase in the total number of PFC, implies that they do not lack a receptor for LPS.

The additional finding that PPD does not increase the Br.MRBC response also strongly suggests that the defective response to Br.MRBC cannot be simply attributed to a lack of an LPS receptor, making it more probable that the SJL has a functional B-cell defect.

We conclude that it is possible that SJL and DBA mice lack Ly-1 B cells, and that the SJL mouse is also unable to respond normally to LPS. It may be unable to respond to other B-cell differentiation signals although preliminary experiments suggest that it responds normally to TRF. Using SJL recombinant inbred lines it should be possible to analyse the defective responses observed in these animals and distinguish between some possible explanations for their impaired B-cell activity.

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